THE

BOTANICAL GAZETTE

MAY 1918

MASS MUTATIONS AND TWIN HYBRIDS OF OENOTHERA GRANDIFLORA AIT.

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(WITH SIX FIGURES)

Under the name of mass mutation, BARTLETT has described a new phenomenon observed by him in *Oenothera pratincola* and *O. Reynoldsii*. Ordinarily, mutations occur in the species of *Oenothera* in about 1 per cent or less of the offspring of self-fertilized individuals, just as they do in the cases of *Linaria* and *Chrysanthemum* and in horticultural instances. In the species studied by BARTLETT (1, 2), about one-half or even a larger number of the offspring were seen to deviate from the parental type in a particular direction. These are called mass mutations; they may appear in the same sowings with normal mutations in other directions.

Oenothera pratincola has produced four mass mutants: mut. *formosa, albicans, revoluta,* and *setacea; O. Reynoldsii* two, mut. *semialta* and *debilis.* BARTLETT has pointed out that the phenomenon bears a certain degree of resemblance to Mendelian segregation, and assumes that the fundamental mutation possibly occurred in only one of the two gametes in a generation preceding the one in which the diversity becomes manifest (2).

Guided by these principles, I have studied the phenomenon of mass mutation in *Oenothera grandiflora* in connection with its ability to produce twin hybrids in certain crosses. .This form of splitting in the first generation after a cross was first discovered

in *O. Lamarckiana* (**6, 9**), but was shown by DAVIS (**3**) to occur in *O. grandiflora* also. I found that *the twin hybrids may be considered as a consequence of the mass mutation*, the mutated gametes producing one of the twins and the typical sexual cells the other. This conception evidently may be applied to *O. Lamarckiana* and make some previous hypotheses superfluous,[1] but this point must be reserved for another article.

I shall first describe my cultures and crosses of *O. grandiflora* in a purely empirical way and afterward discuss their results in connection with those of BARTLETT.

A. MUTATIONS OF O. GRANDIFLORA

One of the last days of September 1912 I visited with BARTLETT a station of *O. grandiflora* in the neighborhood of Castleberry, Alabama. It was on the border of a cornfield situated along the railroad. The station seemed to us to be pure, since no other species of the same group could be discovered either in the field itself or in its neighborhood. The number of specimens was small, but had been very large some years ago, when the field was not cultivated. A few specimens bore ripe capsules, which we collected. From their seeds I started 10 pure strains. One of them was continued through four succeeding generations (1913–1916), whereas the others were abandoned as soon as they proved to contain in the main the same derivatives.

This race produced in my garden three mutations, two of which were observed in every generation, but the third was very rare, occurring only once.[2] All of them were constant in their progeny. I shall call them *ochracea*, characterized by broad and pale leaves, mostly weak and of a low stature (fig. 1); *lorea*, with almost linear leaves and somewhat narrower petals (fig. 2); and *gigas*, with stout stems, broad leaves and flower buds, large flowers, and 28 chromo-

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[1] See *Gruppenweise Artbildung*. The conception of RENNER that the twins, and with them all mutability, might be the effect of a hypothetical hybrid condition of *O. Lamarckiana*, runs in some respects parallel to this view, but is contradicted by it on its main points. See Zeitschr. Ind. Abst. und Vererbungs. **16**: 279–284. 1916.

[2] All of the seeds for the different cultures were soaked in water under a pressure of 8 atmospheres during about 48 hours, and then sown at 30° C. in the greenhouse, so as to induce the most complete germination.

somes in its nuclei.   As was to be expected, besides this *gigas* there was also found a *semigigas*, but since it was wholly sterile, little weight can be attached to it.   GATES (**10**) observed a dwarf mutant of *O. grandiflora*, but no dwarf occurred among my cultures.

The pedigree of the whole culture is as follows.   All fecundations were pure self-fertilizations, made by myself.

First generation	Second generation	Third generation	Fourth generation
1912 1913	1914	1915	1916

Alabama——grandiflora ⎰
  ⎱ ... ⎰ ochracea ———ochracea———ochracea
  lorea ————lorea ————lorea

  grandiflora — ⎰ ochracea
    lorea

    grandiflora — ⎰ lorea
      grandiflora
      ochracea

    semigigas

    gigas ————⎰ gigas
      gigas-lorea
      gigas-ochracea
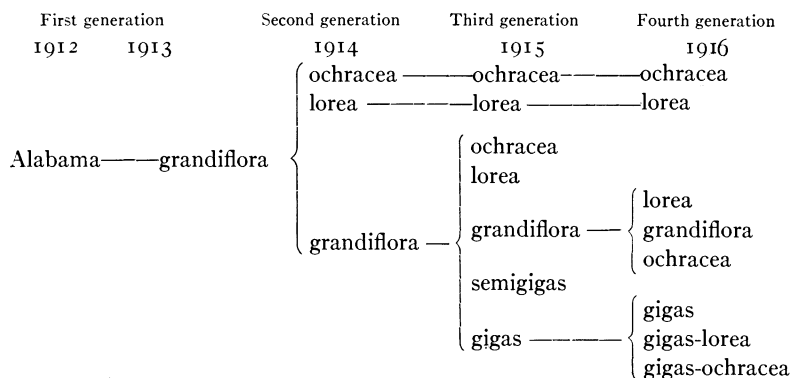
The numbers of specimens and the percentages of the splitting in this pedigree are shown in table I.

TABLE I

	Generation	Number of specimens	Percentage ochracea	Percentage lorea
grandiflora..	1	30	....	....
" ..	2	1476	(20)	2
" ..	3	1180	44	1
" ..	4	53	(15)	4
gigas.......	2	123	1	2.5
ochracea...	2	380	uniform	....
" ...	3	58	"	....
lorea.......	2	160	....	uniform
"	3	61	....	"

The control lines were derived from different specimens, grown in 1913 from the seed of Castleberry.   They yielded the same two main mutants as given in table I.   In the spring of 1914, however, before I discovered the presence of mutants, I had observed that a large number of the seedlings were very weak, dying off during the

first few weeks after being planted out in the boxes.   Most, if not all, of these must have been *ochracea*, and the percentage of 20 for this mutant, found in August during the period of flowering, must have been far too small.   For this reason it is put in parentheses, and the next year I tried to get a more reliable counting.

The seedlings of five self-fertilized plants of 1914 were planted out in boxes as carefully as possible, and before any essential loss
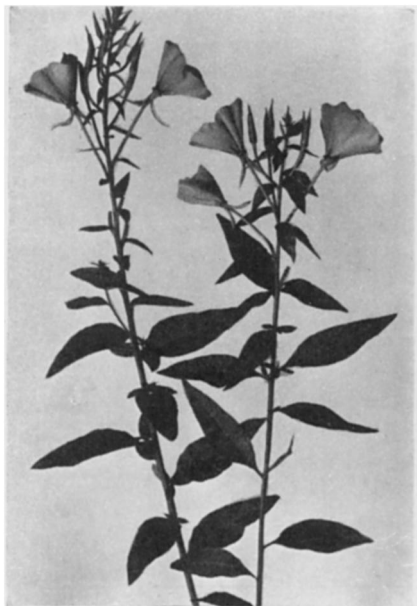
FIG. 1   FIG. 2

FIGS. 1, 2.—Fig. 1, *Oenothera grandiflora* Ait. from Castleberry, Alabama; and to right, *O. grandiflora* mut. *ochracea*, August 1915; fig. 2, *O. grandiflora* mut. *lorea*, August 1915.

was noted.   They were kept in the greenhouse and counted out at the end of April.   At that time some few were dead and decayed; others were dead but could still be judged.   Since I had observed the boxes almost daily, it had been ascertained that it was always the pale ones which died, whereas the green seedlings grew without trouble.   Thus I was confident that the dead had been *ochracea*, as well as the surviving pale ones.   The number of the decayed was

derived from the number of specimens at the time of planting out, minus the number of survivals. The results of the count on April 25, 1915, are given in table II.

TABLE II

PARENT	TOTALS	GRANDI-FLORA	OCHRACEA		LOREA
			Living	Dead	
No. 1........	340	162	89	88	1
" 2........	360	202	93	63	2
" 3........	120	74	29	16	1
" 4........	240	137	45	55	3
" 5........	120	75	25	15	5
Total......	1180	650	518		12
Percentage.	....	55	44		1

The seedlings of the remaining five parents of 1914 were not counted in April, but at the time of flowering in August. Each of the groups yielded a large number of *ochracea* and one or more *lorea*, but the percentage for the first was now only 12. This figure is evidently due to the losses mentioned, since even during the summer usually many specimens of the type of *ochracea* are lost on account of their weakness. In 1916 I got about the same percentage at the time of flowering, but did not estimate the losses during the spring.

For this reason I repeated the sowing in the spring of 1917 with the preserved seeds of the same self-fertilized individual of 1915, taking every possible care to avoid the presumed losses. I planted out 70 seedlings, only one of which died; 20 were found in May to be *ochracea*, giving a total percentage of 30. This figure, therefore, should be substituted in table I for the 15 per cent given for the fourth generation.

Moreover, in 1917 I sowed the seeds of four other self-fertilized individuals of 1915, taking the same precautions. The culture embraced 224 seedlings, of which 8 per cent were pale and weak and died soon after being transplanted, while 31 per cent were recognized in May as *ochracea*. This gives a total of 39 per cent, which corresponds to the figures found in the best of the previous trials.

The percentages in April, as well as those found in August, show that the coefficient of mutation for *ochracea* is wholly different from that for *lorea* and from the ordinary coefficients for the mutability of *O. Lamarckiana*, *O. biennis*, and other species. The average of the three figures for *ochracea* given in table I is 26 per cent, and this figure is somewhat too low on account of the losses mentioned. It is evident, however, that it differs from normal coefficients of mutability in the same way as the mass mutations of BARTLETT, and that the production of mut. *ochracea* from *O. grandiflora* must be considered as another instance of this phenomenon. Here *the mass mutation is repeated in the succeeding generations of the pure line, and, in addition, mutations into lorea and gigas occur in the usual way.*

O. grandiflora mut. *ochracea.*—This species is well known as more strictly annual than any other of the same group. In spring it hardly makes any rosettes of radical leaves, but at once produces its stem. So did all my mutants, but especially the *ochracea* begins to make its stem when still very young and before being planted out. Its foliage is yellowish green, running parallel in this respect to *O. suaveolens* mut. *lutescens* (7). Even as in this last one, the leaves are strikingly broader and somewhat shorter than in the parent species. This insufficiency of the green color causes the young plants to stay behind the normal ones in their development, and by June they are much weaker. Afterward the new leaves assume a darker green, and in the fall the difference is often very small. The weakness remains, however, and the stature is low during the flowering period, reaching only 50 cm. in the beginning of July, when the normal plants are 70–80 cm. in height.

Most of the chlorophyll is developed along the veins. The teeth along the margin usually have red tips. The branches stand out from the stem at wide angles, sometimes almost horizontally. The spikes are loose, but the flowers are large and provided with a rich supply of good pollen; the fruits are cylindrical and often thin. These differences are small, apart from the color, but they are very constant. In fig. 1 they do not show so strikingly as they do on the beds. In consequence of the pale green color of the leaves the stems are thin and their wood is insufficiently developed; they are

often seen to decay, beginning in the lower part of the stem. Many specimens are lost from this cause during the summer.

As given in table I, I cultivated the *ochracea* through three succeeding generations, starting from the mutants of 1914. Two of them were self-fertilized; one yielded a progeny of 50 specimens, which constituted a uniform lot from the first beginning until the end of September. Among these I chose one of the strongest for self-fertilization, and had in 1916 from it a third generation of 58 plants, all resembling their parent. Most of them have flowered.

The other specimens of 1914 yielded seeds, some of which were sown in 1915 and some in 1916. In the first year I had 280 specimens, half of which flowered in August and September and were then pulled up, while the remainder flowered for the most part in October. They constituted a uniform lot of widely branched, low plants of pale green color. The culture of 1916 yielded 50 specimens, as pure and uniform as the former.

I crossed the mut. *ochracea* with the parent species in order to study its hereditary character. I made the crosses in 1915 and got the following progeny in 1916:

	grandiflora	ochracea	lorea	Sum
O. ochracea×grandiflora.......	41	18	1	60
O. grandiflora×ochracea.......	35	16	0	51
Total....................	76	34	1	111
Percentage..............	68	31	1	

The two reciprocal crosses gave evidently the same result, showing that both parents are isogamic in respect to their differential character. For this reason I repeated the sowing in 1917, transplanting the young seedlings after counting them, and determined the percentage of dying individuals besides that of the living *ochracea*. I found for two crosses of *O. ochracea×grandiflora* on May 12, 23 per cent dead seedlings and 35–27 per cent living *ochracea* in a total of 226; means 17 and 31 per cent, together 48 per cent. From the reciprocal cross I had only a small culture of 55 seedlings, among which, however, none died in early youth, while the percentage of the living *ochracea* was 40. The figure for *ochracea* is smaller than the highest one after self-fertilization

(44 per cent), but this obviously resulted from a loss of some pale
individuals, which died off in early youth. The figure of 31 per
cent was determined in June and should rather be compared with
the percentages after self-fertilization determined at the flowering
period (15–20 per cent). The *lorea* seedling was evidently due to
a mutation, even as after self-fertilization.

O. grandiflora mut. *lorea.*—This mutant is characterized by its
very narrow, almost linear foliage throughout its whole develop-
ment. The leaves are dark green. The stature is almost the same
as in the species, although at the end it is 2–3 dm. lower. Our
climate, which is hardly favorable for the Alabama species, is still
less so for this mutant. Not rarely the spikes miscarry, and bare
anthers are of quite common occurrence. Especially in 1915 I
found, during the whole summer, scarcely enough pollen for self-
fertilization and some few crosses. The flowers are somewhat
smaller and the petals less broad than in the parent species, and the
fruits are thinner and more cylindrical. These differences are
small, however, and probably a result of the insufficient nourish-
ment by the narrow leaves. This latter character is always sharp
and clear, and no intermediates have been observed. From two
self-fertilized mutants of 1914 I cultivated a second generation, and
from one of them I derived in 1916 the third one. They were uni-
form lots and strikingly different from the original species. They
embraced in 1915 in the first instance 60 specimens, all of which
flowered, and in the second about 100 seedlings, which were thrown
away as soon as their uniformity was beyond doubt. The third
generation in 1916 consisted of 61 plants, almost all of which
flowered and resembled their parent.

I crossed *O. lorea* with *O. grandiflora* in 1915, but could not find
pollen for the reciprocal cross. In June 1916 I had among 59 indi-
viduals 35 *grandiflora*, 15 *ochracea*, and 9 *lorea*, giving about 60, 25,
and 15 per cent. The figure for *ochracea* is too low, since some
seedlings were yellow and died in the seedpan, but it coincides
sufficiently with the coefficient of mutation from the parent species
as determined in the summer (15–20 per cent in table I). That for
lorea is more reliable, since no losses could interfere here. It must
be considered as due to the combination of all the mutated pollen

grains of *grandiflora* with *lorea* egg cells. It points to a high amount of mutated sexual cells, but my cultures were too small and too few to justify a further discussion of this interesting point.

I have also crossed the two mutants with one another. The results were as follows in June 1916:

	grandiflora	ochracea	lorea	Total
O. ochracea×lorea............	22	8	0	30
O. lorea×ochracea............	31	23	1	55
Total...................	53	31	1	85
Percentage..............	62	37	1	

The results of the reciprocal crosses may be assumed to mean the same hereditary conditions, even as in the crosses of the pale mutant with the species. The specimen of *lorea* seems to be due to a corresponding mutation in the *ochracea*, showing that this mutability is not as wholly absent here as the results of self-fertilization seemed to indicate.

In all these crosses the *lorea* marks must be assumed to be recessive to the *grandiflora* character. I have not made any second generations to decide this question, but the results of my crosses with allied species will fill up this gap and show that in crosses with *lorea* this type is split off, as a rule, in the second generation in proportions which correspond to the law of Mendel.

O. grandiflora mut. *gigas* (fig. 3) occurred in one specimen among the 1180 plants of my cultures of 1915, pointing to a coefficient of mutation of 0.1 per cent. This mutant attracted my attention in May and was planted separately with some other seemingly aberrant specimens. It opened its first flowers in the middle of August. They were strikingly larger, with broad, thick petals, a thicker tube of the calyx, thick filaments, anthers, and lobes of the stigma, and a rich supply of pollen. The flower buds were almost conical and the pollen was rich in quadrilateral grains, one of the characters of the *gigas* mutants of allied species. The nuclei of the young buds were investigated by my assistant Mr. C. VAN OVEREEM, who also counted the chromosomes in the young roots of the seedlings of the following year. The number was invariably 28, showing the

perfect analogy of this beautiful form with *O. Lamarckiana* mut. *gigas* and other giant mutants.

From the self-fertilized seeds of this mutant I had a bed of 123 plants in 1916. They were uniform, with the exception of some specimens of *lòrea* and one *ochracea*. By May all of them had
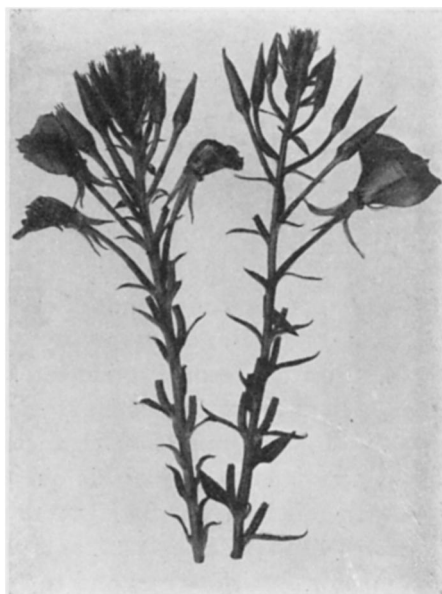

FIG. 3   FIG. 4

FIGS. 3, 4.—Fig. 3, *O. grandiflora* mut. *gigas*, August 1915; flowering spike for comparison with fig. 1; *a*, opening flower bud of *O.* mut. *gigas; b*, flower of *O. grandiflora*, deprived of petals, for comparison with flower on spike of mut. *gigas; c*, opening flower bud of *O. grandiflora; d*, buds for next day's flowers of *O.* mut. *gigas; e*, same of *O.* mut. *grandiflora*; fig. 4, *O. biennis*×*O. grandiflora*, August 1915; to right *laeta;* to left *velutina.*

broader and thicker leaves than *O. grandiflora*, which was cultivated next to it for comparison under exactly the same conditions. The leaves of the young plants in June were 7 cm. broad, 20 cm. long, and a deep, downy green. In July the height was 60–70 cm., but the differences remained the same and very striking, the leaves of *O. grandiflora* being clearer green and only 4 cm. broad. The stems

were much stouter than in the species. During the flowering period the height of the plants exceeded that of the species only a little, but all organs were much stouter. The internodes were shorter and the number of leaves correspondingly larger. Over one-half of the whole culture have flowered, the remainder being pulled out earlier because unexpectedly the crowding of the plants became dangerous. It favors in this mutant, as in the species, the rotting of the stems.

In September I made the following measurements: height 2 m.; leaves of the upper part of the stem 5×15 cm. as compared with 3.5×12 cm., in *O. grandiflora;* petals 4.5 mm. as compared with 4.0×4.2 cm.; tube of calyx 4×50 mm. as compared with 2.5× 35 mm.; flower buds 1.2×4 cm. as compared with 0.8×3.5 cm.; apex of petals with two deep incisions, which, in *O. grandiflora,* are often hardly perceptible; lobes of stigma and filaments of stamens much thicker than in the species. All these characters were very striking on the bed and made the culture one of the most showy of my garden, but the ramification was spare in the mutant; in the species it is ordinarily very rich.

The seeds of mut. *gigas* are about double the size of those of the species. I determined the amount of germs per hundred seeds for three self-fertilized specimens of my culture of 1916, and found 75–88 and 89 with an average of 84 per cent. This is only a little higher than the average for *O. grandiflora* itself, 75 per cent (4). In the roots of one of the three specimens mentioned the chromosomes had been counted by my assistant Mr. C. VAN OVEREEM; their number was 28, as in other instances.

It should be mentioned that the *lorea* and *ochracea* mutants from *gigas* had stout flower buds and large flowers like their sisters, and therefore must be considered as *O. grandiflora gigas lorea* and *O. grandiflora gigas ochracea.*

O. grandiflora mut. *semigigas.*—This mutant of 1915 differs the same way from the species as did the *gigas.* I did not find any striking difference between the two before the fruits ripened. They were stout in *gigas,* but small and thin in the other mutant, which for this reason could not be considered as true *gigas,* but evidently constituted only a *semigigas.* No fertile seeds could be obtained.

Since the occurrence of a mutant *gigas* gives full right to the expectation of mutants of the type *semigigas* with 21 chromosomes, I find no difficulty in the determination of the described specimen, but its value is only of a confirmatory nature.

B.   TWIN HYBRIDS OF O. GRANDIFLORA

One of the most interesting peculiarities of *O. grandiflora* is the production of twin hybrids in certain crosses, analogous to the twins of *O. Lamarckiana*. This splitting was discovered by DAVIS (3) and since confirmed by my own experiments (4). The analogy is very close. All those species which split *O. Lamarckiana* into the twins *laeta* and *velutina* provoke the same phenomenon in *O. grandiflora*. Moreover, *O. biennis Chicago*, when used as a female parent in the crosses, splits both of them into *laxa* and *densa*. In their characters the twins of both species resemble each other so closely as to be easily identified, although it is evident that they cannot agree in all their characters. In those of *O. grandiflora* the differentiating marks are not so sharp as in the twins of *O. Lamarckiana*, and it is sometimes difficult to recognize them in the first culture which offers them. As soon as a second generation is grown, however, all doubts disappear.

The species which split *O. Lamarckiana* into *laeta* and *velutina* are *O. biennis*, *O. syrticola* (*muricata*), and *O. suaveolens* when used as female parents; *O. biennis Chicago*, when its pollen is used; and *O. Cockerelli* in both reciprocal crosses. *O. biennis Chicago* fecundated by *O. Lamarckiana* produces the twins *laxa* and *densa*. All these instances are duplicated by the analogous crosses of *O. grandiflora*. Moreover, *O. Hookeri* produces twins in the reciprocal crosses with *O. Lamarckiana* and also with *O. grandiflora*, but the results of these crosses are of a more complicated nature, and therefore will not be dealt with in this article. Table III gives a list of my crosses, together with their main results.

In *O. suaveolens*×*grandiflora* 18 per cent of yellow specimens appeared; in the other crosses, however, only the twins mentioned appeared. If we sum up the figures for *laeta* and *velutina* and take their mean, we find 52 per cent *laeta* and 46 per cent *velutina*, showing that the figures do not deviate essentially from equality for the

two groups.   Mutants were rare in these cultures.   Among the *laeta* of the first cross an *ochracea* and a *lorea* were seen, and among its *velutina* a *sulfurea*.   Moreover, a *lorea* appeared in the second generation of the *laeta* of *O. lorea*×*Cockerelli*.   The table proves the complete analogy between the splitting phenomena of *O. grandiflora* and *O. Lamarckiana*.

TABLE III

TWIN HYBRIDS OF *O. grandiflora*

CROSS	FIRST GENERATION		SECOND GENERATION	
	Percentage laeta	Percentage velutina	Percentage laeta	Percentage velutina
	A. laeta and *velutina*			
O. biennis×grandiflora...........	90	10	uniform	uniform
O. syrticola×grandiflora*........	47	53	"	"
O. syrticola×grandiflora.........	42	58	"	"
O. suaveolens×grandiflora........	61	21	....	....
O. Cockerelli×grandiflora........	33	67	{10 laeta {90 velutina	uniform
O. Cockerelli×grandiflora........	28	72	....	"
O. grandiflora×Cockerelli........	52	48	{83 laeta {17 velutina	"
O. lorea×Cockerelli.............	60	40	{76 laeta {22 velutina	"
O. lorea×Cockerelli	40	57	....	....
O. grandiflora×Chicago	70	30	uniform	uniform
	B. densa and *laxa*			
	densa	laxa	densa	laxa
O. Chicago×grandiflora..........	83	17	uniform	uniform
O. Chicago×grandiflora..........	75	25	"	"

* The third generation continued uniform.

O. biennis×grandiflora.—I made this cross in 1914 and culti-vated the first generation in 1915.   It embraced 60 plants, almost all of which flowered in July and August.   In the beginning of the flowering period I noticed the presence of two distinct types.   The uppermost leaf beneath the spike was broad in *laeta* (3×10 cm.) and narrow in *velutina* (2×7 cm.), as were also the leaves and bracts. The color was yellowish and pale in the first, but less so in the

second type.   The *velutina* began to flower about a week after the
laeta.   In August the height was 1.50–1.80 m., and the resemblance
of the two types to *O. (biennis×Lamarckiana) laeta* and *velutina*
was very striking, although the plants, as would be expected, were
less stout.   The flower buds of the *velutina* were thick, as usual,
measuring 9×25 mm., as compared with 7×30 mm. for those of
laeta.   The free tips of the calyx were distant in the first, but
pressed against one another in the second hybrid.   The incision
at the top of the petals was deep in the *velutina*, but slight in the
laeta.   The first were more hairy in all parts, especially on the
flower buds and the younger parts of the axis of the spike.   Their
leaves were narrow, kennel-shaped, and smooth.   The apex of the
spike above the flowers was more densely covered by flower buds,
but that of the *laeta* more loose, as shown in fig. 4.   In all these
respects the differences were the same as those between the twins
of *O. biennis×Lamarckiana*.

I fertilized a *laeta* and a *velutina* and had in 1916 a progeny of
63 and 70 plants respectively, most of which flowered.   The off-
spring of the *laeta* contained two mutants, an *ochracea* and a *lorea;*
that of the *velutina* one, a *sulfurea*, with the same pale yellow petals
as in *O. biennis* mut. *sulfurea*.   Besides these, each of the cultures
was uniform, resembling the parent in all respects.   The differences
were apparent in the boxes in May, at the time of planting out.

O. syrticola×O. grandiflora.—O. syrticola Bartlett is the *O. muri-
cata* of my *Gruppenweise Artbildung*.   I made two crosses in 1913,
crossing each plant with the pollen of one individual of *O. grandi-
flora*, as usual.   The figures for both cultures are given separately
in table III; one of them was grown in 1914, but the other in 1915.
From the first I had a second generation for each of the twins in
1915 and a third in 1916.   They were uniform and resembled their
parents.   The size of these cultures was 4 and 49 for the *laeta*, but
61 and 70 for the *velutina*, which had given a better harvest.   One
mutant was observed among the *velutina* of 1915, having linear
leaves and remaining very weak; apart from this the cultures were
strikingly uniform, with the same differences as in the first genera-
tion and almost the same as those between the twins of *O. syrti-
cola×Lamarckiana*.

In this first generation the differences were observed in the beginning of June, since the *velutina* were small plants with narrow kennel-shaped leaves, whereas the *laeta* were stout and had broad, flat leaves.   These differences increased in July and August during the flowering period.   The *laeta* were grass-green, but the *velutina* more gray; these latter had broad flower buds (7×22 mm. as compared with 5×27 mm. in the *laeta*).   The petals were somewhat larger (3 cm.) in the *laeta* and smaller (2 cm.) in the *velutina*.   The fruits were thin in the first named hybrid, but conical in the other.

O. suaveolens × O. grandiflora.—I made this cross in 1915 and cultivated only the first generation.   It consisted of 61 per cent *laeta*, 21 per cent *velutina*, and 18 per cent of a third type, among 69 specimens, most of which have flowered.   The three types were discerned in June and evident in July and August, although the differences between *laeta* and *velutina* were only small.   Height of *laeta* in July 60–80 cm., of *velutina* 40–60 cm., midveins reddish in the first, white in the second.   Leaves 3×10 cm. as compared with 3×15 cm. in July, and 3.5×11 cm. as compared with 2×9 cm. in August.   The flower buds and flowers showed only small differences. The remaining 18 per cent were set off sharply against the rest, and this from the very beginning.   They had the pale color, broad leaves, and low stature of the corresponding mutants of both parents, *O. suaveolens lutescens* and *O. grandiflora ochracea*.   They must evidently be ascribed to the same mutability.   Their flowers were intermediate between those of the parents.   It should be noticed that this is the only case among all the experiments given in table III in which a third type showed itself besides the ordinary twins, apart from stray mutants.[3]   This shows that a special feature of mutability in *O. suaveolens* must be responsible for it.

O. Cockerelli × O. grandiflora.—Since *O. Cockerelli* is an isogamic species, the results of both the reciprocal crosses are the same, with the exception that the hybrids of the cross just named are liable to be more or less pale in their foliage, as is so often the case in crosses in which *O. Cockerelli* is the female parent, as for example in *O. Cockerelli × suaveolens*.   In our case it is the *laeta* which show

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[3] Mutants of the *ochracea* type were seen among the *laeta* of the second generation from *O. grandiflora × Chicago;* see later.

this insufficient development of the chlorophyll, whereas the
velutina is dark green.   The main interest of these crosses lies in
the fact that their *laeta* do not give a uniform progeny, but split
into *laeta* and *velutina*, exactly as in the case of the hybrids of
O. Hookeri and *O. Lamarckiana* (**5**).   The *velutina* constitute con-
stant races in both instances.

I made the cross *O. Cockerelli*×*O. grandiflora* twice, once in
1914 and once in 1915.   They yielded 58 and 64 offspring, among
which 33 and 28 per cent were *laeta* and 67 and 72 per cent *velutina*.
These twins resembled those of *O. Cockerelli*×*O. Lamarckiana*, but
some of the *laeta* had a yellowish green foliage and were more or
less weak in constitution for that reason.   The *laeta* had broad
leaves (4.5×15 cm.), whereas those of the *velutina* were narrow
(3×15 cm.), and the same difference prevailed between the bracts
of the spike.   This character was very conspicuous on the beds,
especially when compared with the cultures of the next generation.
Moreover, I had a lot of *O. syrticola*×*O. grandiflora* at the same
time and found the types of both twins to be essentially the same
as in this cross.

In the second generation the *velutina* were uniform and repeated
the characters of the parent.   The culture embraced 70 flowering
plants.   They were a strikingly uniform lot, and made the dis-
tinction of the two types in the first generation as well as among the
progeny of the *laeta* quite easy.   These latter consisted also of
70 flowering specimens, which were counted in July, shortly before
the opening of the first flowers.   They gave the percentages shown
in table III.

O. grandiflora×*O. Cockerelli.*—Apart from the fact that all the
hybrids are of a normal green color, this cross simply repeats the
reciprocal one.   I crossed two specimens in 1914 and had the first
generation of 80 individuals in 1915.   They showed in July 52 per
cent *laeta* and 48 per cent *velutina*, with the same differences as pre-
viously given and the same resemblance to the twins of *O. syrticola*
×*grandiflora*.   About one-half of the plants flowered, the flower
buds of the *laeta* being relatively thin, but those of the *velutina*
thick (5×20 mm.) and hairy.   The second generation gave a uni-
form lot of 70 flowering plants for the *velutina* and a dimorphic

culture for the *laeta*. In this the types were exactly the same as in the previous year. There were 57 *laeta* and 12 *velutina*, as counted in July, when the differences were most sharp.

O. grandiflora lorea × *O. Cockerelli*.—Apart from the appearance of a few individuals of the *lorea* type, this cross gives the same result as the analogous cross of the species itself, and the hybrids are just the same, not showing the least influence of the almost linear leaves of the mutant mother. I made the cross twice, in 1914 and 1915. The first one gave 80 specimens with 60 per cent *laeta* and 40 per cent *velutina*, but without *lorea*. The second gave 81 flowering plants, among which 23 were *laeta* and 58 *velutina*. Two weak specimens had the leaves of *O. grandiflora lorea*. If we wish to explain their occurrence we must, perhaps, take into consideration that in culture of hybrids of *O. Cockerelli* with other species weak specimens with linear leaves are seen from time to time. In the second generation I expected to find some specimens of *lorea*, but only one appeared among the *laeta*. The culture embraced 64 plants, with 49 *laeta* and 14 *velutina*. I also derived a second generation from the *velutina* of the first; it had the same number of specimens, all of which flowered, but they were wholly uniform and like those just described.

O. grandiflora × *O. biennis Chicago* (cross of 1913).—First generation in 1915 with 40 flowering specimens, among which 12 were weaker than the others from the very beginning, and proved in August, when they flowered, to belong to the type of *velutina*, having narrower leaves. There were still some doubts concerning this identification, but they disappeared when the second generations were cultivated in 1916. These embraced the offspring of two specimens of *laeta*, each consisting of 70 flowering plants, and that of two *velutina*, with 47 and 60 specimens.

The differences were evident by May, since the leaves were broad and clear green in the *laeta*, but narrower and darker in the *velutina*. The *velutina* were quite uniform, but among both groups of *laeta* some specimens showed the broad leaves, pale color, and low stature of the mut. *ochracea* (7 and 12 specimens). The two main types were both intermediate between their parents and much resembled the corresponding twins of *O. Lamarckiana* × *Chicago*.

Leaves of the stem of *laeta* were pale green and broad (4×14 cm.); those of *velutina* dark green and narrow (2.5×12 cm.). Flower buds were shorter and thicker in *velutina* than in *laeta*. The flowers themselves and the fruits were alike in the two twins.

O. biennis Chicago×*O. grandiflora* gives twins which resemble those of *O. biennis Chicago*×*O. Lamarckiana* so closely that there can be no hesitation in identifying them. I made the cross in 1913 on two specimens of the female parent, fertilizing them each with the pollen of one *grandiflora*, but cultivated one offspring in 1914 and 1915, the other in 1915 and 1916, so that in 1915 I had a first and a second generation on the same bed. The results were sensibly the same, as may be seen in table III. The leaves of the *densa* were clearly broader than those of the *laxa*, especially in July and August, when they flowered. In the *densa* the foliage was more dense and the plants more richly branched but lower of stature, and more like the female parent of the cross. The size of my cultures was 70 and 40, mostly flowering plants in the first generations, 60 for each of the second generations of *laxa*, and 70 for each of those of *densa*, making together 370 specimens. The differentiating characters of the first generation were repeated in the uniform lots of the second, where they proved to be clear and sharp.

O. grandiflora ochracea×*O. Cockerelli.*—Since the mass mutant *ochracea* behaves differently from mut. *lorea* in so many respects, I have studied its behavior in this cross and the reciprocal one, in order to see whether the splitting into *laeta* and *velutina* would be repeated or not. I did not find it. Both crosses were made in 1915. In 1916 their progeny embraced 60 and 37 specimens. This latter number was small, because this reciprocal cross produced numerous yellow seedlings, most of which were pale green and did not succeed in developing their first leaves. Only 43 survived in the seedpan, and among these 6 proved still too weak for a normal growth. It is the same phenomenon often seen among the hybrids of *O. Cockerelli* with other pollen. The culture retained some degree of paleness during almost the whole summer. Apart from this, the hybrids of the two reciprocal crosses were the same and constituted one uniform lot. In June the absence of *velutina* was clear; the

hybrids of *ochracea* ✕ *Cockerelli* had broad leaves (6–7 cm.) and were stout green plants, whereas those of the reciprocal cross were still pale. I compared them with the hybrids of *O. grandiflora* ✕ *Cockerelli* and with those of *O. grandiflora lorea* ✕ *Cockerelli* which grew quite near to them. In the beginning of August they began to flower and almost all of the plants of both cultures reached this phase before the end of the month, reaching a height of 1.50 m. They were uniform groups and in all respects like the *laeta* of the corresponding crosses, with the exception of the paleness of one of the two sets; but this diminished gradually as the summer advanced. The leaves and bracts of the inflorescence were still very broad and flat. There were no specimens like the *velutina* of the crosses with *O. grandiflora* and *O. lorea*.

C. UNIFORM HYBRIDS

O. grandiflora ✕ *O. syrticola.*—The hybrids derived from the pollen of *O. syrticola* (*O. muricata*) have often the type described as *gracilis* in my *Gruppenweise Artbildung*. This is especially the case with those of *O. Lamarckiana*, and the hybrids to be described here simply duplicate these latter. I made the cross twice, in 1913 and 1914, and had the first generations of 80 and 30 plants in 1914 and 1915. From the latter I derived a second generation from two self-fertilized individuals of the first. They were uniform lots when they flowered, embracing 7 and 44 specimens with the slender stature and characteristic foliage and stature of *gracilis*, but many seedlings had been yellow and died before making their leaves, exactly as in the first generation. In this the uniformity of the type was already evident in the beginning of June, before the full development of the stems, by the brownish color of the stems and foliage and the narrow, almost linear, leaves. The resemblance to *O. biennis* ✕ *syrticola* increased during the growth of the stems and the development of the spikes. At the time of flowering the plants measured only 80–120 cm.; their top was curved sideward as in *O. syrticola*; the flowers were small and 3–5 of them opened every evening; lobes of the stigma short and thick; leaves narrow, slightly kennel-shaped, and bluish green. It is easily seen that the characters of the father prevailed in the hybrid.

O. grandiflora×*O. biennis.*—This combination corresponds to the cross *O. Lamarckiana*×*biennis*, which gives uniform hybrids of a type in which the characters of the male parent largely dominate; but the results are very different, as we shall presently see.
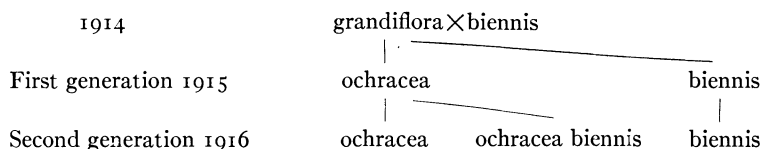
The two parents of the cross have both a large supply of good seeds. The character of *O. Lamarckiana* to produce at least one-half of empty grains is not present in either of them. There is no reason to expect this phenomenon among their crossed seeds, therefore, and as a matter of fact I counted 85 good germs in 100 seeds from this cross; whereas the reciprocal cross, which produces the *laeta* and *velutina* as we have seen, had 79 germs in 100 seeds. The figures do not essentially differ.[4]

I made the cross in 1914, and in 1915 had a set of 54 plants, among which 45, or 83 per cent, resembled their pollen parent in almost all respects, whereas 9, or 17 per cent, repeated the marks described for the mut. *ochracea.* All of the latter and the larger part of the former flowered in August. In the *biennis* type the leaves were narrower (3.0×11 cm.), with reddish midveins; whereas the *ochracea* had the ordinary broad leaves (3.5×11 cm.) and white veins. The stature of the *biennis* exceeded that of the *ochracea* in July by 10–20 cm. (60 cm. as compared with 40–50 cm.). During August these differences gradually increased and the spikes with ripening fruits were compact in the one and loose in the other type, corresponding to those of the pollen parent and of the mutant. The hybrids of the *biennis* type became at the end very stout, reaching almost twice the height of the *ochracea* plants. In 1916 I cultivated a second generation from each of the two types, embracing 70 and 51 specimens, most of which flowered. The offspring of the *biennis* plants were a uniform lot, exactly repeating the characters of their parent; those of the *ochracea* were dimorphic. Some plants made first a rosette of large radical leaves and from this produced a stout stem, whereas the others did not produce a rosette, but at once grew up, causing the stems to be thin and weak. It should be mentioned that the initial rosettes are a character of *biennis*, whereas

[4] My determinations gave, as a mean from 7 countings of lots of 200 seeds each, 75–76 per cent of seeds with normal germs for the cross *O. Lamarckiana*×*biennis* (see 4, p. 268).

O. grandiflora normally produces stems without this preliminary step. In the first generation the differences had been the same in this respect, the *biennis* plants having preparatory rosettes and stout stems, but the *ochracea* lacking these characters. Thus we see that this mark returned in part of the specimens of the second generation. I shall simply call the plants growing up without rosettes *ochracea*, and retain for the others the term "*biennis*,"[5] but both types had the broad, yellowish leaves of the *ochracea* of the first generation, and its loose spikes. I counted in August 33 per cent of the *biennis* and 67 per cent of the *ochracea* type.

Resuming these descriptions we see that the second generation of the first *ochracea* hybrids was constant and like the parent in all respects except the rosette character. This was absent in two-thirds of the specimens, which thereby were just like their parents, but present in one-third, which returned to the mode of growth of the other grandparent. This disposition may be expressed by the following pedigree:

1914   grandiflora✕biennis

First generation 1915   ochracea   biennis

Second generation 1916   ochracea   ochracea biennis   biennis

This pedigree shows the splitting of the *biennis* character in the first and second generations and the constancy of the *ochracea* marks in the second. For the majority of the marks of *ochracea* it runs parallel to the ordinary scheme for the splitting into *laeta* and *velutina*, but for the character of the rosettes it is parallel to that of the *laeta* and *velutina* produced by the crosses between *O. Hookeri* and *O. Lamarckiana*, where the *laeta* is known to split off *velutina* in the succeeding generations. The explanation which offers itself is that the annual growth is here dominant over the

[5] I would have preferred to call them annual and biennial, since one group assumes the annual habit of *O. grandiflora* and the other the biennial growth of *O. biennis* L.; but as I cultivated all of them as annuals, it does not seem advisable to use these terms here. The chosen terms relate obviously to one prominent character; they should not convey the conception that all other characters are the same as in the prototypes of the names.

initial rosettes. These are recessive in part of the first genera-
tion, but return in their offspring in one-third of the specimens, in
about the same way as in the corresponding formula of Mendel. I
have not tried to go deeper into these questions, however, which
touch the mutability of *O. grandiflora* only slightly, but have
limited myself to two further experiments.

O. grandiflora ochracea × *biennis.*—The results of this cross have
been the same as in the pedigree just given, with the exception that
the splitting in the first generation fails. The cross was made in
1914, and the culture of 1915 was a uniform set of 70 plants, most
of which were very weak and died off before flowering. Only 15
reached this stage. They grew up like the normal mutant *ochracea*
and had from the beginning its slender stems, broad and pale
leaves. No specimens of the type of *biennis* were seen. After
self-fertilization a splitting occurred. Some plants were green and
stout; the majority, however, were pale and weak. All of them
had the broad leaves of *ochracea*, but 9 among 80 made vigorous
initial rosettes, whereas the remainder grew up without this prepa-
ration. This gives a percentage of 89 *ochracea* and 11 *biennis*. The
description for both types is the same as in the cross between the
two species.

O. grandiflora lorea × *biennis.*—The narrow leaves of mut. *lorea*
are recessive to the broad form of the leaves of the species. In
other respects the mutant does not seem to differ from it, and thus
I could expect this cross to give almost the same results as the first.
I began the experiment in 1914, and in 1915 had the first generation
with 60 plants, most of which flowered. I counted 9 *ochracea* with-
out rosettes, or 15 per cent; the others were of the biennial type.
Both types agreed in all respects with those of the cross between
the pure species.

The second generation from the *biennis* plants was uniform,
repeating the type of the parent. I had 70 plants, half of which
flowered. They were very stout, and already so in the phase of
rosettes. When flowering, the bed looked almost like pure *O.
biennis* L. No *ochracea* and no *lorea* were seen among them.

The seeds of self-fertilized specimens of *ochracea* of the first gen-
eration produced in 1916, among 67 plants, a very striking splitting

into two types, 57 per cent being stout plants like *biennis* and reaching 1.5 m. in height when they flowered. The others lacked the initial rosettes but were not *ochracea*, evidently being *lorea* with the narrow, dark green leaves of this type. They were far less stout and reached only 1.20 m. in height and flowered some weeks later than the *biennis*. The mut. *lorea* grows always without a preparatory rosette and resembles in this respect the *O. grandiflora*. From this we may conclude that the splitting in our pedigree was exactly the same as that between the two species, with the exception that the *lorea* marks hid those of *ochracea* in the second generation.

I will now resume the results of the three crosses made with the pollen of *O. biennis* L.

TABLE IV

CROSSES OF *O. grandiflora* AND *O. biennis*, MADE IN 1914

CROSS	FIRST GENERATION		SECOND GENERATION FROM OCHRACEA		SECOND GENERATION FROM BIENNIS
	Percentage ochracea	Percentage biennis	Percentage ochracea and lorea	Percentage biennis	
O. grandiflora×biennis	17	83	67 ochracea	33	uniform
O. grandiflora lorea× biennis............	15	85	43 lorea	57	"
O. grandiflora ochracea ×biennis..........	uniform	....	89 ochracea	11	....

If we assume that in *O. grandiflora* the mass mutation into *ochracea* takes place at the time of synapsis, and that the egg cells are therefore mutated before fecundation, we may deduce from table IV that normal egg cells of the species, after fecundation by *O. biennis* L., give uniform hybrids of the *biennis* type, whereas the mutated egg cells reproduce the type of *ochracea*. This would explain the dimorphous condition, where uniformity would otherwise be expected.

O. grandiflora×O. suaveolens.—I made this cross on two specimens in 1915, but got a very small harvest of seeds, yielding only 8 and 13 seedlings. In June the same two types were seen as in the cross of *O. grandiflora×biennis*. I counted 2 and 4 *ochracea*

with broad leaves, a low stature, and weak constitution, and with
stems without preparatory rosettes.   Among the remaining plants
6 and 8 were intermediate between the two parents, with stout,
sparely branched stems and dark green leaves of an intermediate
form.   Besides these there was one *lorea*.   All of these plants
flowered in August, and showed in their flowers intermediate char-
acters; but I have not continued the experiment.

D.   FIRST GENERATION OF CROSSES WITH LAMARCKIANA

Both *O. grandiflora* and *O. Lamarckiana* produce twin hybrids
in a number of crosses.   If they are fertilized among themselves,
therefore, combinations of these twins may be expected.   Moreover,
it is known that from crossed seeds and from seeds of hybrids the
same mutations may arise as from the parent species.   In this way
I observed two hybrids with the characters of *gigas*, some with those
of the dwarfs, and a third type of doubtful relations.   These
mutants are rare, however, whereas the products of the splitting
were observed in all my experiments.

For my crosses I have not only used the two species themselves,
but also one of the mutants of each of them, *O. Lamarckiana nanella*
and *O. grandiflora lorea*.   Their special characters were latent in the
first generation, and the results of their crosses were identical with
those of the species.   They simply give a confirmation of the main
result.   This consisted in the appearance of three types, which it
seems desirable to distinguish by special names.   I shall call these
triple hybrids *ovata*, *lutea*, and *brunnea*, in connection with their most
striking features.   The mutant previously mentioned, for which I
have not succeeded in studying the identity with one of the mutants
of the parents, I shall call, for convenience, *contraria*.   It seems
destined to play only a subordinate rôle in the discussion concerning
the splitting which produces the triple hybrids.

O. hybr. *ovata* is seen almost always in the largest numbers.   It
is stout and richly branched, with broad leaves of pure green and
dense spikes of large flowers.   In spring it makes a large rosette,
like those of *O. Lamarckiana*, and from its center produces a vigor-
ous stem in June.   The foliage is that of *O. grandiflora*, and the
leaves in the middle part of the stem are hairy, show some bubbles,

and measure approximately 4×17 cm. The combination of this foliage with the thick stems and branches of *O. Lamarckiana* constitutes the most striking mark; the hybrid is obviously intermediate between its parents. The flower buds resemble those of *Lamarckiana* and have a tinge of brown, which is subjected to some amount of fluctuating variability. The flowers are large and the

FIG. 5   FIG. 6

FIGS. 5, 6.—Fig. 5, *O. grandiflora*×*O. Lamarckiana* mut. *nanella:* branches of triple hybrids shortly before flowering; *O. ovata* (about 50 per cent); *L. lutea*, and *B. brunnea* (each about 25 per cent of offspring); fig. 6, *O. grandiflora*×*O. Lamarckiana* mut. *nanella:* flowering spikes (deprived of flowers just open), showing characteristic position of flower buds; to left, *O.* hybr. *lutea;* to right, *O.* hybr. *ovata.*

petals cover one another by the margins, but this mark is also variable, and sometimes the petals are seen to have a wedge-shaped base. The fruits are stout and of an intermediate form.

O. hybr. *lutea* differs little from *ovata* in form, but its color is strikingly yellowish instead of pure green. This is seen in the foliage and very evident in the flower buds, but may disappear when the season advances. The leaves are almost as large. I measured

strictly comparable instances for comparison with the figures just given and found 4.5×14 cm., that is, a little broader and shorter than *ovata*. The spike is destitute of red color, or almost so, in all its parts, and this is often the most striking mark. The flower buds are pale and stand off at wide angles from the axis. The flowers are large and the petals have usually a broad base, as in *O. Lamarckiana*. The stems are not as stout nor as richly branched as in *ovata*, but this is probably due to the less amount of chlorophyll. The fruits are like those of the other type.

O. hybr. *brunnea* is a very striking form, especially when cultivated in large numbers of the second generation, which is uniform. It is as high as *ovata*, but less stout; its branches are more erect, its flowers and fruits erect and almost pressed against the stem. The leaves are smooth and narrow, measuring approximately 1.5×8.5 cm. (as compared with 4×17 and 4.5×14 in the two others) and the stem and foliage are brownish, contrasting sharply with the two other types. Even in early youth the differences are sharp enough, although some individuals may remain doubtful, especially when the space at their disposition is not sufficient, but the flowering spikes make all doubts disappear.

O. hybr. mut. *contraria* resembles the *brunnea*, but has larger leaves, measuring approximately 2.5×10 cm., and the color is less brown. The flower buds are thinner and yellowish. It looks like a different combination of the marks of the triple hybrids. Perhaps it is related to *O. Lamarckiana* mut. *oblonga*.

O. hybr. mut. *gigas.*—This occurred in the first generation of a cross between *O. lorea* and *O. Lamarckiana*, and in another between *O. grandiflora* and *O. nanella*. Both were recognized by their broad flower buds, resembling those of *O. Lamarckiana gigas*. The first was a stout plant 1.5 m. high, but little branched, with broad and thick leaves, a short and thick calyx tube, and short and thick fruits. In its other marks it belonged to the type *ovata*. Its pollen consisted almost entirely of quadrangular grains, which were almost completely fertile. Moreover, it was fertile after self-fertilization. Its seeds contained 89 germs in 100 grains. Evidently it was a *gigas* and not a *semigigas*. The other mutant was a *lutea* with thick flower buds. It produced with its own pollen only one fertile

seed, which germinated in 1916. Unfortunately this seedling was attacked by some disease, but it flowered in September with the buds, flowers, and pollen of a pure *gigas*, and showed 28 chromo-somes in the nuclei of its roots in preparations made for me by my assistant Mr. C. VAN OVEREEM.

O. hybr. mut. *nanella* were dwarfs like the hybrid dwarfs of *O. Lamarckiana*. They appeared in the second generation of *lutea* specimens from crosses between *O. Lamarckiana*×*lorea* and *O. lorea*×*nanella*.

I made my crosses in different years and cultivated about 30 or 60 offspring of each. I counted them in July at the beginning of the flowering period, when the characters were most sharp and no doubtful specimens remained. I made one cross first in 1913, and the others in the two following years, so as to have cultures of the second generation along with the trials of the first. This, of course, is the best means of thoroughly comparing the types during the tests.

The size of the cultures is too small to give reliable proportions for each of them. Their aim is to show that the three types arise from every combination without exception, and that the mutants arise only occasionally. The size of the whole group, however, is large enough to warrant the reliability of the average proportions. In calculating these I have reckoned the *gigas* plants with the *ovata* and the *lutea* respectively, on the ground of their general appearance. The *contraria* were calculated separately. No other types occurred, especially no *lorea*, no *ochracea*, no dwarfs, and none of the special types afforded by later generations.

Some details may be given now concerning these experiments.

O. grandiflora×*O. Lamarckiana.*—Cross of 1914 between two specimens of my races. I cultivated 60 offspring until July, but retained only one-half of them during August and September. This half had been planted on a bed in April, before the distinguishing marks were clear. In July I counted 30 *ovata*, 11 *lutea*, and 18 *brunnea*, but could not distinguish the *contraria*. For this reason only the results of the counting on the bed in August have been given in table V. The three types were exactly the same as in the other cultures. Of each of them one plant has been fertilized in order to study their second generations in 1916.

O. grandiflora lorea×*Lamarckiana* (cross of 1914).—In July I had on the bed 10 *ovata*, 12 *lutea*, 8 *brunnea*, and 3 doubtful ones, and in the box 25 *ovata*, 9 *lutea*, and 6 *brunnea*. All of the first and the larger part of the second culture were annual. I retained only those on the bed and controlled their numbers during August, when they flowered, and then found that the doubtful specimens belonged to the type of *contraria*. One specimen of each type has been fertilized and has yielded a second generation in 1916.

TABLE V

O. grandiflora×*O. Lamarckiana* AND DERIVATIVES; FIRST GENERATION

CROSS	YEAR OF CULTURE.	TRIPLE HYBRIDS			MUTANTS		TOTAL
		ovata	lutea	brunnea	con-traria	gigas	
O. grandiflora×Lamarckiana	1915	12	7	8	3	0	30
O. grandiflora lorea×La-marckiana	1915	10	12	8	3	0	33
O. grandiflora lorea×La-marckiana	1916	29	13	17	0	1	60
O. Lamarckiana×grandiflora	1915	22	3	5	0	0	30
O. Lamarckiana×grandiflora lorea	1915	21	4	5	0	0	30
O. grandiflora×nanella	1914	35	12	7	0	0	54
O. grandiflora×nanella	1915	11	2	6	9	1	29
O. grandiflora lorea×nanella	1915	23	2	5	0	0	30
O. grandiflora lorea×nanella	1916	22	25	13	0	0	60
Total		185	80	74	15	2	356
Percentage		52	23	21	4		

A second cross was made in 1915, with the second generation of *lorea*. The culture embraced 60 plants, almost all of which flowered, and which could easily be counted in August. No *contraria* was observed during the period of flowering, but a mutant *gigas* appeared, as has been described. I repeated the counting of this group at different periods, in order to be sure that the same figures were obtained. The plants reached a height of 1.5 m. about the middle of August.

O. Lamarckiana×*grandiflora* (cross of 1913).—Seeds sown in 1915, after a first trial in 1914. Besides the plants mentioned in the table, I had another set, which contained some *brunnea* but no *lutea;* it was thrown away in July. The plants on the bed flowered in August.

O. Lamarckiana×*grandiflora lorea.*—Culture of 61 plants, of which only one-half had been planted out on the bed and flowered in August. The other half consisted in July of 22 *ovata*, 4 *lutea*, and 5 *brunnea*, giving almost exactly the same proportions as those on the bed.

O. grandiflora×*O. Lamarckiana nanella.*—For this and the next crosses the dwarfs of the same race were used, as for almost all my previous crosses with dwarfs. I made the cross on two specimens of *grandiflora* in 1913 and sowed the seeds of one of them in 1914 and of the other in 1915. The first culture showed no *contraria;* the second, however, was extraordinarily rich in them. It contained, moreover, the *lutea* specimen with the flowers of a *gigas*.

The group of 1914 was the first of all my cultures to show the splitting. Before June only two types were distinguished, the yellow plants being considered as weak specimens of the main type. About the middle of June, however, they proved to have broader leaves and quite different flower buds, and were considered to constitute a new type. The final proof of this conception was only reached in 1915, when I cultivated the second generation of the three types, and could observe their distinguishing marks on large sets of plants. In 1914 I counted one-half in the box, and the other at different times on the bed; the sum of the two groups is given in the table.

The culture of 1915 confirmed that of 1914, apart from its mutants. I counted 11 *ovata*, 2 *lutea,* and 6 *brunnea* on the bed, besides 17, 5, and 8 of the same types in the box. These latter have not flowered, however, and for this reason are omitted in the table.

O. grandiflora lorea×*O. Lamarckiana nanella.*—I crossed a mutant *lorea* in 1914 and a specimen of the second generation in 1915. The first cross gave, besides the flowering individuals of the table, 39 *ovata*, 6 *lutea*, and 14 *brunnea*, which had not been planted out for lack of space, but confirm the results of the other set. Almost all of the plants of 1915 flowered in August. All these cultures have been conducted after the same principles, and this makes the description of further details quite superfluous.

The current view concerning the mutations of *Oenothera* is that they take place during synapsis and that the sexual cells are in the

mutated condition before the moment of self-fertilization. If we apply this to the mutability of *O. grandiflora*, we may assume that its sexual cells are divided into two main groups, about one-half remaining typical, whereas the other half belong to the type *ochracea*. Therefore the question arises, which of the triple hybrids just described are produced by the typical gametes and which by the mutated ones? In order to answer this question I made some crosses in which I used *O. grandiflora* mut. *ochracea* instead of the species itself. The *ochracea* constitutes a constant and uniform race and must obviously give the same hybrids as the mutated sexual cells of the parent species.

O. grandiflora ochracea × *O. Lamarckiana.*—I made this cross in 1914 and had two sets of seedlings in 1915, one on the bed and the other in the box. The first embraced 28 *ovata* and 2 *lutea*, the second 23 *ovata* with 3 *lutea;* together 56 plants. Those on the bed were left to flower in August and the counting was then repeated. The culture was one of the most beautiful in my garden and no doubt was possible concerning the identity of the types. Notwithstanding this, no *brunnea* and no *contraria* were observed.

O. Lamarckiana × *O. grandiflora ochracea.*—The result was exactly the same as in the reciprocal cross, but the amount of *lutea* was larger (16 specimens among 69). By the end of August almost all the plants had flowered and were carefully compared with the adjoining cultures of the first and second generations of the other crosses. It was quite evident that no *brunnea* and no *contraria* were present. Especially the *brunnea* constitute a type so widely different from the others that no error could be possible.

O. grandiflora ochracea × *O. Lamarckiana nanella.*—Cross of 1914; first generation in 1915, embracing, as in other instances, two sets, one in a box kept until the end of July and the other on the bed; observed during the whole period of flowering. There were 25 and 27 *ovata* and 5 and 3 *lutea*, but no *brunnea* nor *contraria*.

A résumé of these facts, confining the observations to those made in August at the time of flowering, is shown in table VI.

The conclusion is evident that the gametes of *O. grandiflora ochracea* produce, in their crosses with *O. Lamarckiana*, only two types, *ovata* and *lutea*. These are exactly the same, in all respects,

as the corresponding hybrids between the parent species. No *brunnea* and no *contraria* were observed. The size of the cultures fully warrants these conclusions, but is not large enough to give reliable percentage figures. From these facts it is evident that among the triple hybrids of *O. grandiflora* × *O. Lamarckiana* one type, *brunnea*, is produced only by the non-mutated gametes of the first named parent, whereas another type, *lutea*, is produced exactly by the mutated ones. If we assume that one-half of the

TABLE VI

CROSSES OF *O. grandiflora ochracea*

Cross	Culture	ovata	lutea	brunnea	contraria	Total
O. grandiflora ochracea× Lamarckiana..........	1915	28	2	0	0	30
O. Lamarckiana × grandi-flora ochracea.........	1916	53	16	0	0	69
O. grandiflora ochracea× Lamarckiana nanella....	1915	25	5	0	0	30
Total...............	....	106	23	0	0	129

gametes of *grandiflora* are unchanged and the other half changed into *ochracea*, one-half of the hybrids must result from the first group and the other half from the second. This shows that the pure and the *ochracea* gametes must produce each for one-half *ovata* and for the other their special hybrid. The figures, calculated in table VI, indicate 52 per cent *ovata*, 23 per cent *lutea*, and 21 per cent *brunnea*, and this corresponds as exactly as might be expected to our explanation. Thus we find:

$$\text{O. grandiflora} \times \text{Lamarckiana} = \begin{cases} 50 \text{ per cent pure} \times \text{Lamarckiana} = 25 \text{ per cent} \\ \text{ovata} + 25 \text{ per cent brunnea} \\ 50 \text{ per cent ochracea} \times \text{Lamarckiana} = 25 \text{ per} \\ \text{cent ovata} + 25 \text{ per cent lutea} \end{cases}$$

This formula may be considered to explain the empirical results of our table, since it gives 50 per cent *ovata* and 25 per cent of each of the other hybrids. The empirical figures were 52, 23, and 21 per cent, as just mentioned.

We may go still one step farther and introduce into our consideration the property of *Lamarckiana* to produce the twin hybrids,

laeta and *velutina*.   These are found, on the average, in about equal numbers.   Our formula now becomes:

O.   grandiflora×La-
   marckiana=
$$
\begin{cases}
\text{50 per cent pure} & \begin{cases} \times\text{laeta} &= 25 \text{ per cent ovata} \\ \times\text{velutina} = 25 \text{ per cent brunnea} \end{cases} \\
\\
\text{50 per cent ochracea} & \begin{cases} \times\text{laeta} &= 25 \text{ per cent ovata} \\ \times\text{velutina} = 25 \text{ per cent lutea} \end{cases}
\end{cases}
$$

It is easily seen that this formula opens a deeper insight into the whole phenomenon of twin and triple hybrids.   This point will be discussed further at the end of this paper.

O. *Lamarckiana lata*×*O. grandiflora*.—I made this cross twice in 1914 and 1915 and cultivated the first generation in 1915 and 1916, respectively.   In the boxes it was clear that besides the hybrids described for the parent species, specimens with the type of *O. lata* were present.   They had the broad leaves with rounded tops which are so characteristic of this mutant.   They were planted separately and developed their typical marks during the summer. Their stems remained low and flexible, the foliage was dense, the petioles short, the blades full of bubbles and paler green than in the mutant from *Lamarckiana*.   The flowers and fruits were almost like those of this mutant, but there was plenty of pollen, and the artificial self-fertilization gave a good supply of seeds.   I counted (in 1915) 18 *lata* among 30 plants, and in the next year 24 among 76; together 42 among 106, or about 40 per cent, a figure which does not differ essentially from the hereditary percentages of *O. Lamarckiana* mut. *lata*.   The remaining plants were mostly (41) *ovata*, with some *lutea* and some *brunnea*, some dwarfs, and some other mutants of different types.   Thus we see that this cross gave exactly the results that might be expected.

In 1916 I sowed the seeds of three self-fertilized specimens of the *lata* type.   The cultures showed the same splitting and the same types as in the first generation after the cross.   I counted the *lata* in May and found 13, 15, and 19 per cent, and the *ovata* in May and August; they amounted to 35–51 per cent among 234 individuals. The remainder were partly *lutea* and *brunnea* and partly mutants of different types.   Self-fertilized specimens of *lata* from crosses with *O. Hookeri, O. Cockerelli,* and *O. biennis Chicago* have given analo-

gous splittings, and the experiments just described simply confirm the conclusions drawn from them (5, pp. 252, 254, 255).

E. SECOND GENERATION OF CROSSES WITH O. LAMARCKIANA

As is well known, the twin hybrids from crosses of *O. Lamarcki-ana* are constant in their progeny, with the exception of the *laeta* from the crosses with *O. Hookeri*, which splits into *laeta* and *velutina* in the succeeding generations. For this reason I wanted to know whether the triple hybrids just described would be constant after self-fertilization or split. I found *that none of them split off one of the two others*, and in so far they were constant. On the other hand, some secondary marks, which were not observed in the first generation, turned up in the second, and thus the constancy was not absolute. Since these splittings had no significance for the main object of my study, I have not followed them up.

TABLE VII

CULTURES OF SECOND GENERATION

Cross	Cross	Second generation	ovata	lutea	brunnea	contraria
O. grandiflora×Lamarcki-ana..................	1914	1916	+	+	+	+
O. grandiflora lorea×La-marckiana	1914	1916	+	+	+	+
O. Lamarckiana×grandi-flora..................	1913	1916	+	−	+	−
O. Lamarckiana×grandi-flora lorea.............	1914	1916	+	+	+	−
O. grandiflora×nanella....	1913	1915	+	+	+	−
O. grandiflora×nanella....	1913	1916	+	+	+	+
O. grandiflora lorea×nan-ella	1914	1916	+	+	+	−
O. nanella×grandiflora....	1913	1915	+	−	−	−
O. lata×grandiflora........	1914	1916	+	−	−	−
O. grandiflora ochracea×Lamarckiana...........	1914	1916	+	+	−	−
O. grandiflora ochracea×nanella................	1914	1916	+	+	−	−

In respect to the third generation, it was to be expected that it would simply confirm the results of the second, and so I have limited myself to one culture for each of the three main types and to some few for the secondary combinations. Table VII gives a list of my cultures of the second generation. They embraced with some few

exceptions 60–70 specimens each, and almost all of these have flowered. As the types of the triple hybrids were exactly the same as in the first generation, no special descriptions will be necessary. In this table + means that a second generation of the type mentioned in the heading above it has been cultivated, whereas − indicates that no culture of the type has been tried.

A third generation of *ovata* was cultivated for *O. nanella* ✕ *grandiflora*. From the reciprocal cross *lutea* and *brunnea* were continued during one generation more. I shall treat the crosses of *O. grandiflora lorea* separately, since they split off this mutant type, and deal first with the others. Among the special combinations appearing in the second generation there were three which could clearly and easily be distinguished, but only two of them were frequent. I shall designate them by the letters *R*, *T*, and *L*. Among these, *R* was a return to the rapid production of a stem, without preparatory rosette of radical leaves, which is so characteristic a mark of *O. grandiflora*, but which was always dormant in the first generation. The plants were usually slender and small, the leaves broad and dark green, and they flowered one or two weeks before their stouter sisters. In July they reached 10–30 cm. more in height than these, but during the flowering period they were overgrown by them. Their flowers showed the same forms. The progeny of the type *R* was uniformly so. The type *T* was easily recognized by its truncate flower buds; these are conical in the parental species and in the triple hybrids. The flowers were correspondingly smaller. The leaves were almost like those of *ovata*, but strikingly broader in their upper half. The height and stature were also the same. In their progeny they repeated their characters exactly, but split off some specimens of the type *R*. Type *T* was remarkably rich in the production of pitchers. The type *L* combined the characters of the hybrid called *lutea* with the slender stature, rich branching, and thin flower buds of *O. grandiflora*. It produced some specimens of *R* among its progeny, which was otherwise uniform. A continued study of these and other hybrid types of *O. grandiflora* would probably offer the material for an analysis of the characters of this species. In counting my cultures of the offspring of self-fertilized *ovata* at the beginning of the flowering period, I found the figures as given in table VIII.

The percentage figure for type R conforms to the formula of Mendel for monohybrids, assuming the initial rosettes to be dominant over their absence, but in the cross of *O. grandiflora* with *O. biennis* the reverse was the case. Possibly the linkage was

TABLE VIII

SECOND GENERATION OF *ovata*

Cross	Second generation	ovata	R	T	L	Total
O. grandiflora×Lamarckiana........	1916	46	14	9	0	69
O. Lamarckiana×grandiflora........	1916	60	19	2	0	81
O. grandiflora×nanella..............	1915	59	13	4	1	77
O. grandiflora×nanella..............	1916	50	16	0	0	66
O. nanella×grandiflora..............	1915	44	6	0	1	51
O. lata×grandiflora.................	1916	45	16	2	0	63
O. grandiflora ochracea×Lamarckiana	1916	39	27	6	0	72
O. grandiflora ochracea×nanella.....	1916	53	23	1	0	77
Total........................	....	396	134	24	2	556
Percentage....................	....	71	24	4.5	0.5	......

TABLE IX

THIRD GENERATION FROM *ovata*

Cross	Second genera-tion	Third generation				Total
		ovata	R	T	L	
O. nanella×grandiflora..............	ovata	37	25	0	0	62
" "	R	0	29	0	0	29
O. grandiflora×nanella..............	R	0	20	0	0	20
" "	R	0	79	0	0	79
" "	T	0	31	36	0	67
" "	T	0	3	45	0	48
" "	L	0	6	0	52	58
" "	L	0	8	0	62	70
Total........................	....	37	201	81	114	433

different in the two instances. In these second generations I self-fertilized some individuals and cultivated their offspring in 1916. Table IX gives their offspring. This gives a percentage of 42 for the splitting off of type R from the others, and this figure seems to be analogous to the one deduced from our previous table (24 per cent). Apart from this splitting, the types T and L had a uniform progeny. The third generation of *ovata* repeated the constitution of the second in its most essential points.

The offspring of self-fertilized *lutea* consisted of this form and type *R*, but none of the other secondary types appeared among them.   Table X is made up in the same way as that for *ovata*.   In one instance a third generation, derived from two successive generations of *lutea*, was also studied.

TABLE X

SECOND AND THIRD GENERATION OF *lutea* CULTIVATED IN 1916

Cross	Generation	lutea	R	Total
O. grandiflora×Lamarckiana.......	2	44	25	69
O. grandiflora×nanella............	2	33	12	45
O. grandiflora ochracea×Lamarcki-				
ana............................	2	45	11	56
O. grandiflora ochracea×nanella....	2	33	15	48
O. grandiflora×nanella............	3	28	51	79
Total.........................	....	183	114	297
Percentage...................	....	62	38	....

The percentage for *R* is 38 per cent, coinciding sufficiently with those in the two previous cases (24 per cent and 42 per cent).

As shown in table VII, I have self-fertilized specimens of *brunnea* in 7 different cultures of the first generation.   I cultivated 60–70 offspring from each of them and studied them all during their whole lifetime until the first fruits began to ripen in August.   One of them flowered in 1915, and one plant was fertilized so as to have a second generation of *brunnea* in 1916.   It embraced 70 flowering plants. All these cultures were uniform; they produced no *R* and no others, and not even a *lorea* in the beds derived from crosses of this mutant. The same table shows three self-fertilized specimens of *contraria*, the offspring of which was studied, in 60, 70, and 72 flowering individuals in 1916.   In one case there were three doubtful specimens like type *R*, but apart from these the cultures were uniform and like their parents.

We now come to the crosses of the mutant *lorea*.   The special mark, consisting in the almost linear leaves, was latent in the first generation, but was seen to return in the second, whenever specimens of *ovata* or *lutea* are self-fertilized.   The *brunnea*, however, did not split them off, as we have just seen.   I made the following cultures (table XI) and counted them in the same way as previously

described, in the beginning of the flowering period.   The percentage figures for the appearance of types R and T correspond to those derived from our table for *ovata*, which were 24 and 4.5.   The figure for *lorea* is a low one, but in the cross *O. grandiflora×biennis* we have seen that 43 per cent *lorea* were split off in the second

TABLE XI

SECOND GENERATION OF CROSSES OF *O. grandiflora lorea;* CULTURES OF 1916

Cross	Second genera-tion	ovata	lutea	R	T	nanella	lorea	Total
O. grandiflora lorea×La-marckiana	ovata	54	. . . . . .	25	7	o	6	92
O. grandiflora lorea×La-marckiana	lutea	. . . . . .	51	4	o	o	28	83
O. Lamarckiana×grandi-flora lorea	ovata	58	. . . . . .	26	10	o	2	96
O. Lamarckiana×grandi-flora lorea	lutea	. . . . . .	68	2	o	2	9	81
O. grandiflora lorea×nan-ella	ovata	51	. . . . . .	15	3	o	2	71
O. grandiflora lorea×nan-ella	lutea	. . . . . .	50	6	o	1	9	66
Total	. . . . . .	163	169	78	20	3	56	489
Percentage	. . . . . .	68		16	4	1	11	. . . . . .

generation.   The question whether this phenomenon conforms to the formula of Mendel for monohybrids remains to be answered by more extensive cultures.

F.   MASS MUTATIONS, CONSIDERED AS SECONDARY MUTATIONS

After describing the facts observed in my cultures and experi-ments, we may now proceed to the discussion of the principle of BARTLETT, already quoted.   He assumed that a fundamental mutation occurred in one of the two gametes in a generation pre-ceding that in which the mass mutation appeared.   We are not concerned, however, with the question whether all instances of mass mutation are due to the same internal processes, but only with the problem of explaining the production of mut. *ochracea* from *O. grandiflora* by means of BARTLETT'S suggestion.

In order to proceed in an empirical way, and to rely as much as possible on analogy with well ascertained facts, I shall start from

a consideration of the mutation of *O. Lamarckiana gigas* into its dwarf mutants (8). These spring from the self-fertilized strain of *O. gigas* in about 1–2 per cent of the offspring of every generation, and have done so since the very origin of the parent form. Artificially crossed with their parent, they produce hybrids of high stature, which are not externally distinguishable from *O. gigas* itself, and which split, in the next generation, into three types, according to the formula of Mendel for the monohybrids. Assuming, as is now generally conceded, that mutations take place before fecundation, we can easily see that the gametes of *O. gigas* which mutated into *nanella* must for some part be united in fecundation with normal sexual cells. Such combinations must produce half mutants, as I called them in my book *Gruppenweise Artbildung*,[6] or mutant hybrids, as they have since been called (8, p. 345), and these will split in the next generation into about one-fourth dwarfs, one-fourth high and normal *gigas*, and one-half new mutant hybrids. The latter will continue to reproduce the splitting in the succeeding generations, and this may obviously be repeated during an unlimited number of successive years.

If we now suppose that, by means of some contrivance, the dwarfs and the constant high specimens were yearly eliminated before flowering, we should have a race which would produce in every generation about one-fourth dwarfs. The phenomenon would then be an instance of mass mutation, and we may choose it as the prototype from which to explain our observations on *O. grandiflora*. From our point of view the splitting would be a repeated appearance of the dwarf mutants, due to the original mutation of one gamete. For this reason we shall call it a *secondary mutation*.

Let us now consider the strain of *O. grandiflora*, found in 1912 near Castleberry, Alabama, as such a mutant hybrid, originally produced by the mutation of a sexual cell into *ochracea* and its copulation with a normal gamete of the strain. Whether this initial mutation took place a few or many years before 1912 is of course without interest for this discussion. It may even be older

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[6] The case is especially clear in the instance of *O. Lamarckiana semigigas*, where the half mutants with their 21 chromosomes are obviously the result of the copulation of normal gametes with others mutated into *gigas*.

than the species itself. We further assume a close analogy with the mutant hybrids of *O. gigas nanella*. This conduces to the expectation of three types in every generation, namely, constant *ochracea*, constant *grandiflora*, and hybrid mutants.

The *ochracea* are our secondary mutants; they were seen to arise in my cultures constantly during the three generations of my experiments, and every time in large numbers. The mutant hybrids are the apparently normal specimens of *grandiflora* of my strain; they repeat the splitting in every generation, but no constant *grandiflora* have been found, since all the specimens tried reproduced the mass mutation. Here, therefore, we have to introduce another principle. This is the assumption of a lethal factor. MORGAN and his students have discovered the presence of four such units in their experiments with *Drosophila*, and from their studies we know exactly what to expect from them (**11**). I have already proposed this principle for the explanation of the empty seeds of *O. Lamarckiana* (**4**), and we may apply it here in the same way. I determined the amount of barren grains among the seeds of my strain of *O. grandiflora* and found 12–41 per cent, with an average of 25 per cent for the harvest of 8 self-fertilized plants of different generations (**4,** table on p. 245). Now our argument led us to expect 25 per cent of constant specimens, and the hypothesis that these are killed within the seeds by some lethal factor would at once explain their absence and the presence of the barren grains.

By means of this hypothesis the conception of our strain of *O. grandiflora* as a hybrid mutant now becomes complete. It starts from two succeeding initial mutations in sexual cells, which copulated with normal ones. One of these was the mutation into a weak, yellowish *ochracea*, the other was the production of a lethal factor, linked to the non-mutated gametes. This linkage must be assumed to be so complete as not to interfere with the applicability of Mendel's formula for monohybrids.

The presence of the lethal factor in both the gametes of a copulation kills the germ after some time, but the presence of the same factor in only one of the two gametes leaves them viable. This latter proposition is proved by numerous crosses between species with barren grains and those without the factor in question. Such seeds are always capable of normal development.

The supposed initial mutations of our strain, therefore, must have produced half mutants, the gametes of which split in every generation into about equal parts of potential *grandiflora* with the lethal factor, and into viable *ochracea*. The fecundation must then produce one-fourth of germs of *grandiflora* with the double lethal factor and thereby doomed to die off within the seed; one-fourth of viable but weak *ochracea*, which will be constant in their progeny; and one-half of hybrids between the two mutants, in which the qualities of the type of the species will be dominant, whereas the lethal factor must be recessive.

Among the living seedlings the proportion of green hybrid mutants and yellowish *ochracea* must therefore be 2:1, and the average figure for the latter was 26 per cent, although this was somewhat too low on account of the loss of part of the yellow seedlings in early youth. Artificial crosses between the hybrid mutants and the *ochracea* should give about 50 per cent of either type. I found for both the reciprocal crosses about 34 per cent, but the figure was depressed from the same cause. A repetition of these experiments, excluding the influence of these losses, is proposed; it is expected to give a fuller proof.

We assume the supposed initial mutations to have been analogous to the mutations into *lorea* and *gigas*, which may still be observed to occur in my garden. New mutations into *ochracea* may occur also, but they must evidently always escape observation, being different in no respect from the secondary or mass mutation.

BARTLETT has pointed out the analogy between the phenomenon of mass mutation and Mendelian splitting, observing, however (1, p. 452), that "there can be no doubt that mass mutation is not Mendelian segregation, although the two phenomena have points of resemblance." In our instance this resemblance is plain enough, but a splitting is called Mendelian if it is observed among the progeny of hybrids between different species, varieties, or strains, whereas the half mutants are hybrids between mutated and nonmutated sexual cells of the same parent. They are produced by one experimental pure line, whereas real hybrids are the result of the combination of different strains. The hybrid mutants start from a mutation; they can never be made use of as an argument against

the mutation theory.   The names of mass mutation and secondary
mutation, therefore, seem to be very appropriate, indicating, as
they do, the true explanation of the phenomenon.

G.   TWIN HYBRIDS, CONSIDERED AS A RESULT OF MASS MUTATION

In *Gruppenweise Artbildung* (5) I have devoted a large part to
the study of the twin hybrids of *O. Lamarckiana* and its derivatives
in their crosses with other species.   I was convinced that some
relation must exist between the cause of this curious phenomenon
and the high degree of mutability of the species.   I supposed this
internal cause, whatever it might be, to be responsible in a large
degree, not for the mutability itself, since this is not a special trait
of the *Lamarckiana*, but for the exceptionally high degree of develop-
ment of the quality in that species.

Later investigations of different authors, and especially those of
RENNER, have confirmed this conception, since they do not offer
an explanation of the problem involved on the basis of other excep-
tional qualities of my plant.   The experiments described for *O.*
grandiflora, however, prove that there is still another relation, since
the twins may be considered as the result of the fecundation of
sexual cells which are, for a large part, in the condition of mutated
gametes.   It is evident that in crosses these latter may give differ-
ent hybrids from those of the normal gametes of the same parent.
I shall now try to show that the results of my crosses confirm this
view in almost all their details.

We have to start from the assumption that the mass mutations
take place in the same numerical proportions as those required by
the formula of Mendel for monohybrids; in other words, that the
two kinds of gametes are produced in equal numbers and among
the pollen as well as among the egg cells.   Fecundation with a
different species must then produce two kinds of hybrids, each of
them in about 50 per cent of the offspring.   Our table for the pro-
duction of *laeta* and *velutina* in such crosses gave on the average
52 per cent for the first and 46 per cent for the latter, and thus fully
confirms our conception.   When the crosses are repeated with mut.
ochracea instead of the type of the species itself, no twins must be
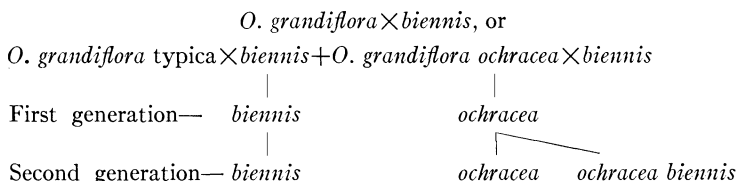the result, but only uniform hybrids of the type corresponding with

the *laeta*.   Until now I have tried only one instance, *O. grandiflora* ×
O. Cockerelli and its reciprocal.   They produced only one of the
twins, namely, *laeta*, and thus confirm our view.   Other combina-
tions should be studied for the same purpose.

If Mendel's law were applicable to the production of the twins,
these must split after self-fertilization into three or more types.
Our table shows that this is not, or at least not always, the case.
The *velutina* never split, nor do the *laeta* of *O. biennis*, *O. syrticola*,
and *O. biennis Chicago* produce a splitting.   Only those of *O. Cock-*
erelli show this phenomenon, but here it is limited to the repetition
of the mass mutation into *velutina*.   From these facts we must con-
clude that the hereditary factors involved are not in the condition
required by Mendel's laws.   In *Gruppenweise Artbildung* I have
called this deviating condition labile, leaving the question open
whether it may be determined by means of linkage or otherwise.

The cross of the pollen of *O. grandiflora* with the female gamete
of *O. biennis Chicago* produces twins which are quite different from
the *laeta* and *velutina*, and are therefore called *densa* and *laxa*.
Formerly I assumed this difference to be due to the splitting of
another factor, but my results with *O. grandiflora*, in connection
with the appreciation of the complicated nature of so many muta-
tions (7), open the prospect of considering it as due to the same
unit, only under the influence of different linkage.   In fact, if we
assume the pollen of *O. grandiflora* to be dimorphous before fecun-
dation, two types of hybrids must be expected in this cross as well
as in others.   This conception simplifies the problem, although it
does not offer a direct proof against the presence of a special splitting
factor for *densa* and *laxa*.   These twins are constant in their pro-
geny, even as the *laeta* and *velutina* just considered, and thereby
indicate the same special condition of the factors involved.   When-
ever the differentiating characters of the twins are recessive to
those of the other parent, the twins must show the same external
marks.   The hybrids will then be uniform instead of dimorphic,
as, for example, in our experiment with *O. grandiflora* ×*O. syrticola*
(*muricata*).

The cross *O. grandiflora* ×*O. biennis* gave a dimorphic progeny,
which may evidently be ascribed to the presence of mutated and

normal gametes in the first species. If we assume this to be the true interpretation, the pedigree may be written in this form:

O. grandiflora×*biennis*, or

O. grandiflora typica×*biennis*+*O. grandiflora ochracea*×*biennis*

First generation—   *biennis*   *ochracea*

Second generation—*biennis*   *ochracea*   *ochracea biennis*

Read in this way our experiments show that in the normal gametes of *O. grandiflora* the characters of the species are recessive to those of *O. biennis*, and that uniform and constant hybrids are produced. The gametes which repeated the mutation into *ochracea*, on the other hand, possessed dominant characters, and the offspring was hardly distinguishable from normal *ochracea*. But after self-fertilization it split off the *biennis* type, especially the character of producing stout initial rosettes before making a stem, and this splitting seems to conform to Mendel's law for mono-hybrids.

H.   ANALOGY BETWEEN THE TWINS OF O. GRANDIFLORA AND O. LAMARCKIANA

In concluding this article I might point out the striking analogy between the splitting phenomena of *O. grandiflora* and those of *O. Lamarckiana*. Evidently they must be considered as the results of the same internal causes. The chief difference is the absence of a visible mass mutation in the latter species. On the contrary, the amount of barren grains among its seeds is double that of *O. grandiflora*. We are therefore induced to assume a second lethal factor, linked with the characters of *ochracea* and *laeta*, respectively, and killing the *laeta* germs of *O. Lamarckiana*. Or, stating it in other words, we may imagine the factor for weakness, which causes the death of a large part of the *ochracea* mutants after germination, to be replaced in *O. Lamarckiana* by a lethal factor, which kills the corresponding germs before germination.

RENNER (**12**) has proposed an explanation which in some respects parallels the views developed in this article, but, as I have

already explained, it differs mainly in the conception of the first origin of *O. Lamarckiana*. RENNER considers this species to be a hybrid between two previously existing types, corresponding to *laeta* and *velutina*, and sees in this hybrid condition the cause of its mutability. The analogy with *O. grandiflora* leads us, however, to consider this "hybrid condition" not as the cause but as a result of the mutability. Elsewhere I have shown that his conception leads to contradictions and requires too many additional hypotheses, even without considering the analogy with *O. grandiflora* (4). A detailed criticism of RENNER'S views from this latter standpoint, however, must be postponed until another opportunity.

Summary

1. *Oenothera grandiflora* Aiton from Castleberry, Alabama, splits in my cultures in every generation into two types. One of them consists of strong, green plants of the parent type; the other of weak, yellow individuals, of which only a few are vigorous enough to flower and ripen their seeds. This weak type is called *O. grandiflora* mut. *ochracea*.

2. Besides these it produces other mutants in the ordinary proportions of 0.1–1 per cent, namely, mut. *lorea* with almost linear leaves and mut. *gigas* with 28 chromosomes and the corresponding stoutness of all its organs. These two types are constant from seed, but the *gigas* keeps on mutating into *lorea* and *ochracea*.

3. The crosses among *O. grandiflora*, *O. ochracea*, and *O. lorea* show that these forms are isogamic, the pollen carrying the same hereditary qualities as the egg cells.

4. *O. grandiflora* yields twin hybrids with the same species which produce twins in their combinations with *O. Lamarckiana*. The female organs of *O. biennis*, *O. syrticola* (*muricata*), *O. suaveolens*, the pollen of *O. biennis Chicago*, and both sexes of *O. Cockerelli* split *O. grandiflora* into *laeta* and *velutina*, whereas the cross *O. biennis Chicago*×*grandiflora* yields the twins *densa* and *laxa*. The twins appear, on the average, in about equal numbers. This splitting fails when the crosses are made with mut. *ochracea* instead of *O. grandiflora*. Their progeny is uniform and corresponds, so far as investigated, to the *laeta* among the twins.

5. In other crosses of *O. grandiflora* the hybrids also resemble the corresponding ones of *O. Lamarckiana*. *O. grandiflora* × *syrticola* produces the type *gracilis*, *O. grandiflora* × *biennis* hybrids of the types of *biennis* and *ochracea*, among which the first is constant in its progeny, whereas the second repeats the splitting.

6. In crosses with *O. Lamarckiana*, *O. grandiflora* produces combinations of the two groups of twins. I found three such types. One of them embraces about one-half of the offspring and corresponds to the *laeta;* it is called *ovata*. The two others appear each in one-fourth of the whole culture and are called *lutea* and *brunnea*. The first corresponds to the combination *ochracea* × *velutina*, the second to *grandiflora* × *velutina*. These triple hybrids are constant in their progeny, inasmuch as they do not produce individuals of the other types, but split off some forms which constitute different combinations of the parental characters and partly also of those of the mutants. One of them, lacking the initial rosette of radical leaves, appears in percentage figures which seem to correspond to the formula of Mendel for monohybrids.

7. If the crosses are made with the mutant *lorea*, this character is latent in the first generation and reappears in the second in about one-quarter of the individuals; but this rule shows some exceptions.

8. From these facts, in combination with the occurrence of about 25 per cent of barren grains among the seeds, we arrived at the conclusion that the yearly production of large numbers of *ochracea* is a phenomenon of mass mutation, analogous to the instances described by BARTLETT and due to an initial mutation of the ordinary rare type, followed by secondary mutation in the succeeding generations.

9. This initial mutability of *O. grandiflora* must have yielded, besides the ordinary mutants, hybrid mutants, produced by the combination of a mutated sexual cell with a normal one. If then the offspring of this fecundation is assumed to split in a manner analogous to Mendel's formula for monohybrids, three types must be the result. One of them is the mut. *ochracea*, which is now a secondary mutant; the second is the mutant hybrid of the type of the species, which will repeat the splitting; and the third must be a constant form of the same type. This last does not appear, and

a lethal factor is assumed to answer for this gap.   It must be linked to the otherwise pure *grandiflora* gametes.   It explains the absence of the constant type, together with the presence of a corresponding percentage of empty seeds.

10. In this way the mass mutation as well as the empty grains can be explained by the assumption of two initial mutations of the ordinary type.   One is that into *ochracea*, the other is the origin of a lethal factor linked to the gametes which are not mutated into a weak, yellow form.

11. The twin hybrids, mentioned under 4, must be the result of the same secondary splitting of the gametes.   Those of the *grandiflora* type must yield the *velutina* and the *laxa;* those carrying the characters of *ochracea* must give the *laeta* and the *densa*.

12. The twins produced by the crosses of *O. grandiflora* with *O. biennis*, *O. syrticola*, and *O. biennis Chicago* are constant in their progeny, but the *laeta* from crosses with *O. Cockerelli* repeat the splitting into the types of the twins.

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